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Energy expenditure responses to fasting and overfeeding identify phenotypes associated with weight change

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Abstract: Because it is unknown whether 24-h energy expenditure (EE) responses to dietary extremes will identify phenotypes associated with weight regulation, the aim of this study was to determine whether such responses to fasting or overfeeding are associated with future weight change. The 24-h EE during energy balance, fasting, and four different overfeeding diets with 200% energy requirements was measured in a metabolic chamber in 37 subjects with normal glucose regulation while they resided on our clinical research unit. Diets were given for 24 h each and included the following: 1) low protein (3%), 2) standard (50% carbohydrate, 20% protein), 3) high fat (60%), and 4) high carbohydrate (75%). Participants returned for follow-up 6 months after the initial measures. The decrease in 24-h EE during fasting and the increase with overfeeding were correlated. A larger reduction in EE during fasting, a smaller EE response to low-protein overfeeding, and a larger response to high-carbohydrate overfeeding all correlated with weight gain. The association of the fasting EE response with weight change was not independent from that of low protein in a multivariate model. We identified the following two independent propensities associated with weight gain: a predilection for conserving energy during caloric and protein deprivation and a profligate response to large amounts of carbohydrates.

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Energy expenditure responses to fasting and overfeeding identify phenotypes associated with weight change

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Abbreviations

%EE: percent change in 24-h EE during the dietary intervention

CNP: high-carbohydrate, normal-protein overfeeding with 75% carbohydrate, 5% fat and 20% protein

CRU: clinical research unit

EE: energy expenditure

FNP: high-fat, normal-protein overfeeding with 20% carbohydrate, 60% fat, 20% protein

FST: fasting

FM: fat mass

FFM: fat free mass

LPF: low-protein overfeeding with 51% carbohydrate, 46% fat, 3% protein

OGTT: oral glucose tolerance test

RQ: respiratory quotient

SOF: standard overfeeding with 50% carbohydrate, 30% fat, 20% protein

SPA: spontaneous physical activity

WMD: weight-maintaining diet

1 **ABSTRACT**

2 Because it is unknown if 24-h energy expenditure (EE) responses to dietary extremes will identify
3 phenotypes associated with weight regulation, the aim of this study was to determine whether such
4 responses to fasting or overfeeding associate with future weight change. The 24-h EE during
5 energy balance, fasting and four different overfeeding diets with 200% energy requirements was
6 measured in a metabolic chamber in 37 subjects with normal glucose regulation while they
7 resided on our clinical research unit. Diets were given for 24-h each and included: 1) low-protein
8 (3%), 2) standard (50% carbohydrate, 20% protein), 3) high-fat (60%), and 4) high-carbohydrate
9 (75%). Participants returned for follow-up 6-months after the initial measures. The decrease in
10 24-h EE during fasting and the increase with overfeeding were correlated. A larger reduction in
11 EE during fasting, a smaller EE response to low-protein overfeeding and a larger response to
12 high-carbohydrate overfeeding all correlated with weight gain. The association of the fasting EE
13 response with weight change was not independent from that of low-protein in a multivariate
14 model. We identified two independent propensities associated with weight gain: a predilection
15 for conserving energy during caloric and protein deprivation, and a profligate response to large
16 amounts of carbohydrates.

17

INTRODUCTION

Human overfeeding studies suggest that there is a considerable inter-individual variation in the energy cost of weight gain (1-5). In a prior cross-sectional study, the increase in energy expenditure (EE) with overfeeding and the decrease with fasting were found to be correlated in a small group of 14 male subjects (5). Our group has previously shown that the EE response to overfeeding varies considerably among individuals but is consistent and reproducible within individuals. This individual contribution explains more of the observed variability in the EE changes with overfeeding than changes to the macronutrient content of the diet (6). These studies seem to indicate that phenotypic differences may exist in the EE responses to fasting or overfeeding that may affect susceptibility to weight gain. As overeating or caloric restriction are necessary to alter weight, perturbations in energy balance may be needed to uncover responses that signify an energy conserving physiology versus a physiology that is better able to resist weight gain. We now extend our previous findings by addressing the question of whether this inter-individual variation in EE changes relates to future weight change.

During overfeeding, the metabolic response depends, in part, on the macronutrient composition of the diet in addition to the contribution from inter-individual variation (6). Although it has been proposed that low-protein diets might magnify differences in the propensity to obesity (2; 7), a recent study has shown that the EE response is smaller and fat mass (FM) gain is similar when overeating low protein diets compared to normal protein diets (8). Further, high-carbohydrate diets have been shown to have a greater EE increase during overfeeding compared to high-fat diets (9). In addition, a single, large high-carbohydrate meal has been shown to activate brown adipose tissue (10). Differences in the short-term (24-h) EE response to overeating diets varying in macronutrient content may therefore facilitate identification of human phenotypes with increased susceptibility to future weight gain. We hypothesized that a larger

reduction in EE during fasting and a smaller increase in EE during 24-h of overfeeding would be associated with weight gain at 6 months in free-living, healthy individuals not counseled on any lifestyle changes. In addition, we hypothesized that varying the macronutrient content of the overfeeding diet might identify macronutrient-specific differences in the EE response to overfeeding that would be more strongly associated with future weight change.

RESEARCH DESIGN AND METHODS

Subjects

Volunteers were recruited from the Phoenix, Arizona area between 2007 and 2013, and admitted to our clinical research unit (CRU) for 25 days to participate in an inpatient study exploring the metabolic responses to fasting and overfeeding (ClinicalTrials.gov Identifier: NCT00523627). Among the 59 individuals who completed the baseline admission, thirty-seven had follow-up data for body weight 6 months after discharge and were included in the present analysis (Figure 1). This report represents a pre-planned analysis of an ongoing study when a target sample size of 37 subjects had completed the 6-month follow-up to provide 90% power ($\alpha=0.05$) to detect a simple correlation of 0.5 between percent change in EE with overfeeding or fasting and the primary endpoint of body weight change at follow-up. These 37 individuals did not differ from the larger initial group with regards to demographics, anthropometrics and 24-h EE measures. All subjects were weight stable for at least 6 months and healthy according to history, physical examination, electrocardiogram and laboratory testing. None of the subjects had a vegetarian or gluten-free lifestyle, and none had a known food allergy. All women were premenopausal and not pregnant. All volunteers provided informed, written consent. The experimental protocol was approved by the Institutional Review Board of the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK).

Upon admission, volunteers were given a weight maintaining diet (WMD) consisting of 50% carbohydrates, 30% fats, and 20% proteins with total caloric content based on previously derived equations specific to our CRU that include weight, BMI and sex (11). Morning weight was checked daily and the WMD was adjusted as necessary throughout the stay to maintain a stable weight ($\pm 1\%$). The WMD was given throughout the stay except the days when subjects had 24-h EE assessments. Volunteers were asked to consume all food given, and to only engage in sedentary activities for the duration of their stay on the CRU. Body composition was measured using dual-energy X-ray absorptiometry (DXA) (DPX-1, Lunar Corp, Madison, WI, USA). After 3 days on the WMD, a 75g oral glucose tolerance test (OGTT) was done. Only individuals with normal glucose regulation (12) were eligible to participate. Plasma glucose concentrations were measured using an enzymatic oxygen-rate method (Beckman Glucose Analyzer 2; Beckman Instruments, Brea, CA) ($n=7$) or the comparable Analox GM9 glucose oxidase method (Analox Inst. USA Inc., Lunenburg, MA, USA) ($n=30$).

Energy expenditure measures and dietary interventions

Each volunteer completed seven 24-h EE assessments in a whole-room indirect calorimeter: two eucaloric assessments (EB0 and EB) followed by five EE measures during the dietary interventions described below (Figure 2). There was a 3-day washout period in between each dietary intervention for any residual effects of the 24-h dietary intervention to wane. The average CV of the volunteers' body weight prior to the dietary interventions was $0.94 \pm 0.48\%$, indicating that body weight was stable ($< 1\%$) during the admission period.

For all diets, volunteers were given breakfast at 07:00 and entered the calorimeter one hour later. Further meals were provided inside the calorimeter at 11:00, 16:00, and 19:00 through a two-door airlock. Total energy intake of the 4 meals given during the first eucaloric measurement in the metabolic chamber (EB0) was 80% of the WMD to account for reduced

activity in the calorimeter (13). To increase the precision of the EE measure during energy balance, energy intake during the second eucaloric measurement (EB) was equal to the 24-h EE value measured in EB0. The 24-h EE from this second eucaloric assessment (EB), which was used as the baseline comparator, was then doubled to determine the kilocalories (kcal) given for the subsequent overfeeding diets (200% energy requirements).

Volunteers completed in randomized order five intervention diets, each given for only 24 hours: fasting (FST); low-protein overfeeding with 51% carbohydrate, 46% fat, 3% protein (LPF); standard overfeeding with 50% carbohydrate, 30% fat, 20% protein (SOF); high-fat, normal-protein overfeeding with 20% carbohydrate, 60% fat, 20% protein (FNP); high-carbohydrate, normal-protein overfeeding with 75% carbohydrate, 5% fat and 20% protein (CNP) (Figure 2). Macronutrient composition of each diet was determined using The Food Processor software (ESHA Research, Salem, OR, USA). Subjects returned all uneaten portions to the metabolic kitchen for weighing, so that actual intake by macronutrient could be calculated. Five (2% of total 222 chamber sessions) EE measurements (1 standard, 2 high fat, and 2 high carbohydrate diet) were excluded as less than 95% of food was consumed.

Ambient temperature averaged $23.6 \pm 1.4^{\circ}\text{C}$. The average O_2 consumption and CO_2 production were used to calculate the 24-h EE and respiratory quotient (RQ), as previously described (6). RQ was used as a proxy for the carbohydrate-to-fat oxidation ratio. Quality control tests were done monthly, and demonstrated mean recoveries of $99 \pm 3\%$ ($\text{CV}=3.6\%$) and $98 \pm 3\%$ ($\text{CV}=3.4\%$) for O_2 and CO_2 , respectively. Energy balance was the difference between caloric intake and 24-h EE. Spontaneous physical activity (SPA) was detected by radar sensors and expressed as the percentage of time in which motion was detected.

Follow-up visit

Upon completion of the EE assessments, participants were not provided with any lifestyle counseling and were advised to return to their usual habits. They were, however, provided with the results of their DXA scan and OGTT. Participants were discharged and asked to return at a scheduled 6-month follow up visit for measurement of weight and body composition.

Statistical analysis

Statistical analyses were performed using the procedures of the SAS Institute Inc. (SAS version 9.2; Cary, NC). Alpha was set at 0.05. Data are presented as mean±SD. The Shapiro-Wilk test was used to assess normality of data. Data were scanned for potential outliers using the methods of Grubbs (14), Tukey (15) and the generalized Extreme Studentized Deviate (ESD) test (16). No outliers were identified. Differences between groups were evaluated using Student's *t* test or chi-square analyses for continuous and categorical variables, respectively. Ethnic differences were assessed by one-way ANOVA. To normalize the EE response to body size, the percent change in 24-h EE (%EE) during each dietary intervention was calculated as the difference divided by the 24-h EE during energy balance (EB) and expressed as a percentage:

$$\%EE\ response_{diet}(\%) = \frac{(24\text{-h } EE_{diet} - 24\text{-h } EE_{energy\ balance})}{24\text{-h } EE_{energy\ balance}} \times 100$$

Pearson correlations were used to determine correlations between normally distributed continuous variables, and Spearman correlations were used for non-normally distributed variables. For some analyses, the EE responses to the 4 overfeeding diets were averaged per person to understand general effects of overfeeding. Associations with the response to overfeeding were determined from mixed models accounting for repeated measures and including the variables age, sex, ethnicity, percentage body fat, and diet. Differences between diets were adjusted for multiple comparisons using Tukey's range test.

Significant correlations between EE responses to fasting and overfeeding with weight change were followed-up with regression models to adjust for age, sex, ethnicity, and baseline weight. All results were confirmed using percent weight change per month in place of absolute weight change. Similar models were calculated for the absolute changes in FM and FFM including initial baseline measures as covariates. Multivariate regression models were created to determine independence of the identified associations. Adjusting for SPA did not substantially change any results, thus only findings using unaltered 24-h EE are reported.

RESULTS

Subjects characteristics

General, anthropometric and EE characteristics of the study population during energy balance are shown in Table 1. Body composition, 24-h EE and percent change in EE with fasting or overfeeding did not differ between ethnic groups.

24-hour energy expenditure response to fasting or overfeeding

Compared to energy balance, %EE decreased with fasting ($-8.5\pm5.0\%$; $p<0.001$) and increased with overfeeding (Table 2, Figure 3B). The average percent increase in 24-h EE during the four overfeeding diets ($9.0\pm4.0\%$) correlated with the percent decrease in 24-h EE with fasting ($r=0.55$, $p=0.001$) (Figure 4A). Individually, the percent decrease in 24-h EE with fasting correlated with the percent change in 24-h EE during low-protein overfeeding ($r=0.46$, $p=0.006$) (Figure 4B). The percent increase in 24-h EE during high-carbohydrate overfeeding correlated with the %EE responses to the high-fat, normal-protein ($r=0.53$, $p=0.002$) and standard overfeeding ($r=0.38$, $p=0.02$) diets, as well as with the percent decrease in 24-h EE with fasting ($r=0.40$, $p=0.02$). The mean %EE response to overfeeding (the average of all four diets) was inversely related to percent body fat ($r=-0.43$, $p=0.008$). In a mixed model accounting for

repeated measures, adjusting for age, sex and ethnicity, and including only the four overfeeding diets, diet ($p<0.001$) and percent fat ($\beta=-0.12\%$, $p=0.03$) were independent determinants of %EE.

Determinants of future weight change

Changes in body weight and body composition at follow up (6.5 ± 0.9 months, range: 5.2 to 9.2 months) are shown in Table 1. The variance in weight change at 6 months was normally distributed (Shapiro-Wilk $p=0.44$) around a mean increase of 1.2 ± 4.4 kg (range: -6.1 to 11.2 kg) without any suspected outliers. There was no difference between sexes or ethnicities in body weight change.

A greater reduction in 24-h EE during fasting was associated with weight gain at 6 months ($r=-0.35$, $p=0.04$) (Figure 5A), and this was still true after adjustment for age, sex, ethnicity and baseline weight ($\beta=-0.32$ kg per 1% difference in 24-h EE response, $p=0.05$). Similarly, the %EE response during low-protein overfeeding at baseline was negatively associated with absolute body weight change ($r=-0.55$, $p=0.001$) (Figure 5B), and this held true after adjustment for age, sex, ethnicity and baseline weight ($\beta=-0.42$ kg per 1% increase in 24-h EE response, $p=0.01$). There was no association between change in body weight and the average percent change in 24-h EE during the 3 overfeeding diets with 20% protein content ($r=0.16$, $p=0.35$), nor were the EE responses to standard ($r=0.03$, $p=0.86$) or high-fat, normal-protein overfeeding ($r=0.06$, $p=0.75$) associated with weight change. The EE response to high-carbohydrate, normal-protein overfeeding was positively associated with weight change at follow-up ($r=0.33$, $p=0.05$; $\beta=0.41$ kg per 1% increase in 24-h EE, $p=0.009$ adjusted for age, sex, ethnicity and baseline weight) (Figure 5C). In a multivariate model, both the 24-h EE responses to low-protein ($\beta=-0.44$ kg per 1%-difference in 24-h EE response, $p=0.004$) and high-carbohydrate, normal-protein overfeeding ($\beta=0.38$ kg per 1%-difference in 24-h EE response,

p=0.003), but not the 24-h EE response to fasting ($\beta=-0.15$ kg per 1%-difference in 24-h EE response, $p=0.18$), were independently associated with weight change at follow-up. Results did not change with serial adjustment for age, sex, ethnicity nor baseline weight. The EE response to fasting was only significantly associated with weight change when the 24-h EE response to low-protein overfeeding was removed from the multivariate model.

To further illustrate the independent effects of the EE response to low-protein and high-carbohydrate overfeeding on weight change, we categorized subjects in four subgroups according to the median percent change in 24-h EE during these two overfeeding diets (Figure 6). Subjects with a higher-than-median EE response during high-carbohydrate overfeeding and lower-than-median EE response during low-protein overfeeding ($n=7$) gained more weight compared to those with the opposing EE responses ($n=6$) (mean difference= +7% of their baseline weight, $p=0.007$), despite similar baseline body weight ($p=0.80$). The 24-h EE response to low-protein overfeeding was associated with changes in both FM ($r=-0.48$, $p=0.004$) and FFM ($r=-0.36$, $p=0.04$) at 6 months; however the EE response to high-carbohydrate overfeeding was only associated with FM change ($r=0.37$, $p=0.04$), but not FFM change ($p=0.6$).

The 24-h RQ during fasting ($r=-0.41$, $p=0.01$), but not during any overfeeding diet (all $p>0.2$), was negatively associated with weight change (Figure 5D), and this was still true after adjustment for age, sex, ethnicity and baseline weight ($\beta=-0.56$ kg per 0.01 change in fasting RQ, $p=0.01$). The fasting RQ was associated with the FFM change at 6 months ($r=-0.34$, $p=0.05$), but not with change in FM ($r=-0.26$, $p=0.14$). There was no association between the 24-h RQ and either the percent change in 24-h EE ($p=0.14$) or the absolute 24-h EE ($p=0.23$) during fasting. Neither the EE response to low-protein nor the response to the high-carbohydrate overfeeding diet was correlated with the fasting RQ. In a full model including all observed associations with weight change, only the percent changes in 24-h EE during low-protein

($\beta=-0.46$, $p=0.002$) and high-carbohydrate overfeeding ($\beta=0.39$, $p=0.006$) remained independent predictors of weight change at 6 months. All results were similar if the dataset was limited to only men. All longitudinal results were similar, and often slightly stronger, if percent weight change from baseline weight was substituted for absolute weight change (data not shown).

DISCUSSION

Our results confirm that humans have the ability to respond to overfeeding and fasting with an increase and a decrease in EE, respectively, and that these responses are directly correlated. At baseline, body adiposity was inversely related to the EE response to overfeeding. Individually, four variables related to fasting and overfeeding including a greater decrease in EE with fasting, a smaller response to low-protein overfeeding, a greater EE response to high-carbohydrate overfeeding, and a lower fasting RQ were associated with weight gain at 6 months in free living adults on an ad libitum diet. However, only two independent phenotypes associated with future weight gain emerged including a more energy conserving response to low-protein feeding, during both calorie deficit and caloric excess, and separately, a larger EE response to high-carbohydrate overfeeding.

It is well recognized that EE increases with overfeeding and decreases with fasting (5; 6; 17). In a prior cross-sectional study, these responses to overfeeding and fasting were correlated within individuals implying the possibility of “thrifty” and “spendthrift” phenotypes within the population (5). Recent work from our group found that obese individuals with a more thrifty phenotype, defined by the %EE response to fasting, lost less weight in a carefully controlled inpatient weight loss study with six weeks of 50% caloric restriction (18). We have now confirmed that these %EE responses are correlated, and shown that it is not so much the response to caloric restriction, but rather the response to protein restriction, that defines a “thrifty” phenotype. Consistent with the finding that more thrifty individuals lose less weight during

caloric restriction (18), we observed that free-living individuals with a thrifty phenotype are more likely to gain weight over time. Contrary to expectations, a greater EE response to over-consuming large amounts of carbohydrates, an effect that might be expected to attenuate weight change, was associated with weight gain. Our study differs from many prior studies, which have assessed the impact of long-term underfeeding (19-22) or overfeeding (8; 17; 23-25) with specific diets, in that we were assessing differences in baseline physiology and how such inter-individual differences might interact with typical dietary patterns to influence weight change.

It is known from studies such as the Minnesota experiment and the Biosphere 2 project that prolonged energy restriction leads to adaptive reductions in EE (5; 19-21). Of note, the diets in both of these studies also had a relatively low proportion of protein (<12%). A more recent study investigating the effects of chronic overconsumption of low, normal, and high-protein diets (8) found that FM gain was similar in all 3 groups, although low-protein led to smaller changes in overall weight due to differences in FFM. In our study, both a larger reduction in EE with fasting and a smaller EE response to the low-protein diet despite caloric excess were associated with future weight gain. These responses were correlated and, in a multivariate model, only the low-protein response remained associated with weight change indicating a potential similar underlying physiology. A candidate pathway that might explain these findings is the hepatic response to amino acid deprivation that leads to secretion of fibroblast growth factor 21 (FGF21) (26; 27). Although FGF21 was originally reported to increase with fasting (28-30), a recent study has demonstrated that it is protein restriction, not caloric restriction, that induces increases in circulating FGF21 in rodents and in humans (27). This study also found that FGF21 is required for the EE response to low-protein (27). We observed that the low-protein diet led to the smallest increases, and even decreases, in EE with overfeeding. Other studies have reported that long-term overfeeding is required for any increased, potentially adaptive, EE response to a low-protein diet

(17; 24; 27). As sustained intake of a low-protein diet would be unusual in modern society (31), our results may reflect that those individuals able to increase EE more quickly during even short periods of protein restriction are better able to prevent weight gain.

The increase in EE with overfeeding was greatest with the high-carbohydrate diet, but surprisingly, a larger EE increase with this diet was associated with more weight gain. The underlying physiology behind the larger increase in EE with carbohydrate intake is unknown and may be related to genetic differences, alterations induced by prior dietary choices, i.e. a chronically high-carbohydrate diet prior to admission, or a robust inflammatory response to carbohydrates (32). When subjects are fed an isocaloric high-carbohydrate diet for 2 weeks, those individuals who are more likely to store carbohydrates, rather than oxidize them, gain less FM over time (33), and we may be observing a similar phenotype. Alternatively, a high-carbohydrate meal has been reported to increase brown adipose tissue activity (10), which would lead to increased EE. As a higher EE during energy balance has been associated with greater subsequent *ad libitum* food intake (34; 35), the availability of high carbohydrate foods in a free-living condition may increase EE and subsequently drive further energy intake in the absence of dietary restraint.

Fasting RQ was no longer associated with weight change after accounting for the EE responses to low-protein and high-carbohydrate overfeeding. Thus, the initial simple correlation may be due to confounding or may indicate similar, overlapping physiologic mechanisms with the overfeeding results. The association of greater lipid oxidation with fasting, i.e. a lower RQ, with future weight gain might suggest that a greater reliance on lipid stores during energy restriction is involved in body weight regulation. This finding may be consistent with a phenotype that preferentially oxidizes rather than stores carbohydrates (33) as the increased lipid oxidation during fasting may reflect smaller amounts of glycogen stores. The previously reported

associations of higher carbohydrate oxidation during energy balance with both subsequent increased food intake (36) as well as weight gain (36; 37) are further evidence that phenotypic differences that indicate a preference to oxidize, rather than store, ingested carbohydrates are related to weight gain.

A limitation of our study is the lack of hormonal measures that might explain the underlying mechanisms of the EE changes. Nevertheless, prior results from a subset of these subjects did demonstrate that catecholamine responses were similar for both fasting and the low-protein diet (6). Additional long-term follow-up are needed to determine if the baseline measures of EE are associated with weight changes over longer periods of time (38-41). Subjects were asked to resume their previous lifestyle upon discharge, and none of the subjects reported substantially changing their diet in the intervening period but formal assessments of diet or physical exercise during the follow-up period were not done. This was purposeful, as we wanted to examine the relationship of baseline EE physiology with spontaneous short-term weight change under free-living, unencumbered conditions. In addition, it is possible that the level of physical fitness prior to admission may have contributed to the EE response to overfeeding; however, all subjects were admitted to the clinical research unit at the time of the initial assessment and had similar levels of physical activity during the inpatient stay. Further, adjusting for spontaneous physical activity in our analyses did not impact the results. Although the study includes a small proportion of lean women relative to women classified as obese, all results were similar if the dataset was limited only to men. Even in this relatively small study group, we were able to identify subjects with differing phenotypes defined by their EE response to low protein and high carbohydrate overfeeding, and people with these phenotypes had substantially different changes in body weight at follow-up. Nevertheless, future studies with larger study populations are warranted to replicate and confirm our results.

In summary, we identified a number of metabolic phenotypes correlated with subsequent weight change that condensed into two independent phenotypes: a smaller EE response to low-protein intake and a greater EE increase with high-carbohydrate intake. Based on these results, it is reasonable to hypothesize that the observed inter-individual variation in the EE response to protein restriction constitutes the long sought, but previously unidentified, “thrifty phenotype” that accounts, in part, for the observed inter-individual variation in weight loss during similarly calorically restricted diets. Further, the inter-individual variation in the EE responses to high-carbohydrate intake may account, in part, for the utility some individuals find in eating a carbohydrate-restricted diet to limit weight gain. To conclude, an increased understanding of the phenotypic differences between people in response to over- or undereating may lead to new strategies to prevent weight gain.

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MS and PP are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The

323 contributions of the authors were as follows – MS: Data collection, data analysis and writing of
324 the manuscript; PP: data analysis, revision of the manuscript; NP: study design, writing of the
325 initial clinical protocol when he was employed at the NIDDK-Phoenix in 2006, review of the
326 manuscript; SB: study design, data collection, review of the manuscript; JK: study design, review
327 of the manuscript; MST: study design, data collection, data analysis, writing of the manuscript.
328 None of the authors had any conflicts of interests.

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Table 1. Demographic, anthropometric and metabolic characteristics of the study group during energy balance and at the 6-month follow-up.

	Whole study group	Men	Women	p-value*
	(n=37)	(n=27)	(n=10)	
Ethnicity	7 AA, 11 W, 9 H, 10 NA	2 AA, 8 W, 8 H, 9 NA	5 AA, 3 W, 1 H, 1 NA	0.02 [†]
Age (years)	36.1 ± 9.6 (19.3, 54.1)	36.7 ± 10.3 (19.3, 54.1)	34.7 ± 7.8 (21.3, 44.7)	0.58
Body weight (kg)	77.8 ± 11.8 (56.4, 107.8)	78.4 ± 10.3 (60.6, 103.5)	76.1 ± 15.9 (56.4, 107.8)	0.60
Height (cm)	172.7 ± 6.4 (156.8, 185.0)	175.2 ± 5.1 (161.5, 185.0)	166.1 ± 4.8 (156.8, 170.0)	<0.001
BMI (kg/m²)	26.1 ± 4.0 (18.3, 39.1)	25.6 ± 3.4 (18.3, 33.4)	27.6 ± 5.5 (20.7, 39.1)	0.19
Body fat (%)	28.2 ± 11.4 (6.9, 53.8)	23.4 ± 8.2 (6.9, 36.4)	41.2 ± 8.3 (24.2, 53.8)	<0.001

FM (kg)	22.5 ± 11.2 (4.9, 56.9)	18.8 ± 8.0 (4.9, 33.0)	32.4 ± 13.0 (13.6, 56.9)	<0.001
FFM (kg)	55.3 ± 9.5 (34.2, 79.4)	59.6 ± 6.9 (46.9, 79.4)	43.7 ± 4.2 (34.2, 50.9)	<0.001
Fasting glucose (mg/dL)	92.2 ± 4.6 (80.0, 99.0)	92.1 ± 5.0 (80.0, 99.0)	92.5 ± 3.4 (89.0, 99.0)	0.82
2-h glucose (mg/dL)	102.6 ± 20.2 (46.0, 133.0)	102.9 ± 21.5 (46.0, 133.0)	101.8 ± 17.3 (80.0, 132.0)	0.88
24-h energy intake (kcal/day)	2063 ± 278 (1529, 2645)	2146 ± 247 (1658, 2645)	1838 ± 235 (1529, 2249)	0.002
24-h EE (kcal/day)	2036 ± 281 (1502, 2575)	2116 ± 261 (1616, 2575)	1822 ± 223 (1502, 2290)	0.003
24-h energy balance (kcal/day)	26.4 ± 69.3 (-117, 169)	30.5 ± 69.9 (-117, 159)	15.5 ± 70.1 (-52, 169)	0.57
Body weight change (kg)	1.2 ± 4.4 (-6.1, 11.2)	1.4 ± 4.6 (-6.1, 11.2)	0.8 ± 4.2 (-5.2, 8.4)	0.71

Body weight change (%)	1.5 ± 5.6	1.8 ± 5.8	0.8 ± 5.2	0.63
	(−7.2, 14.1)	(−6.8, 14.1)	(−7.2, 8.6)	
FM change (kg)	0.1 ± 3.7	0.2 ± 4.0	−0.4 ± 2.9	0.66
	(−9.3, 8.6)	(−9.3, 8.6)	(−4.5, 4.7)	
FFM change (kg)	0.7 ± 2.2	0.7 ± 2.1	0.8 ± 2.3	0.96
	−2.6 ± 6.6	(−1.9, 6.6)	(−2.6, 4.1)	

Data are presented as mean ± SD, with minimum and maximum values in parentheses.

* P-values are for differences between men and women as determined by Student’s *t*-test.

† Ethnic differences between genders were assessed by Chi-squared test.

AA, African American; W, White; H, Hispanic; NA, Native American.

EE, energy expenditure during energy balance; FM, fat mass; FFM, fat free mass.

Table 2. Extent of 24-h EE responses during eucaloric feeding, 200% overfeeding with diets varying in macronutrient content, and fasting.

Diet	24-h FQ (ratio)	24-h RQ (ratio)	24-h EE (kcal/day)	Change in 24-h EE (%)	TEF (%)	24-h SPA (%)
Energy balance (EB)	0.86	0.87 ± 0.03	2036 ± 281	N/A	8.4 ± 4.9 [‡]	5.4 ± 3.3
Fasting (FST)	0.71	0.79 ± 0.03 [*]	1857 ± 224 [*]	−8.5 ± 5.0 [‡]	N/A	5.0 ± 3.7
Low protein overfeeding (LPF)	0.85	0.91 ± 0.05 [*]	2093 ± 299 [*]	+2.8 ± 4.9 [‡]	5.7 ± 2.7 [‡]	5.7 ± 4.1
Standard overfeeding (SOF)	0.86	0.89 ± 0.04 [*]	2251 ± 339 [*]	+10.9 ± 5.7 [‡]	9.9 ± 3.3 [‡]	5.9 ± 3.3
High fat overfeeding (HPF)	0.78	0.83 ± 0.04 [*]	2186 ± 319 [*]	+8.7 ± 4.9 [‡]	8.7 ± 3.0 [‡]	5.6 ± 3.7
High carbohydrate overfeeding (CNP)	0.93	0.94 ± 0.05 [*]	2330 ± 321 [*]	+14.4 ± 5.3 [‡]	11.8 ± 3.5 [‡]	6.5 ± 4.2

Data are presented as mean ± SD.

^{*} p<0.05 by Tukey's range test compared to energy balance.

[†] p<0.05 vs. 0

[‡] p<0.0001 vs. 0

EE, energy expenditure; FQ, food quotient; RQ, respiratory quotient; SPA, spontaneous physical activity, TEF, thermic effect of food.

The FQ, i.e. the expected 24-h RQ based on the macronutrients in each diet, was calculated from published equations (6). Percent change in 24-h EE was calculated with respect to the 24-h EE during energy balance. The TEF of each diet was calculated by subtracting the 24-h EE during fasting from the 24-h EE during the relevant dietary intervention, and then expressed as percent of the corresponding total caloric intake.

FIGURE LEGENDS

Figure 1. Flow diagram of participant progress through the study.

Figure 2. Study diagram of the clinical study.

Figure 3. Macronutrient composition of the dietary interventions (A) and related 24-h EE response (B).

Protein, carbohydrate and fat content of the diets are expressed in grams based on a representative diet for an individual requiring 2000 kcal for energy balance and 4000 kcal for overfeeding (Panel A). The 24-h EE response to each dietary intervention is expressed as percent change compared to the 24-h EE measured during energy balance (Panel B). Error bars represent mean with SD.

EB, energy balance; FST, fasting; LPF, low-protein overfeeding with 51% carbohydrate, 46% fat, 3% protein; SOF, standard overfeeding with 50% carbohydrate, 30% fat and 20% protein; FNP, high-fat, normal-protein overfeeding with 20% carbohydrate, 60% fat and 20% protein; CNP, high-carbohydrate, normal-protein overfeeding with 75% carbohydrate, 5% fat and 20% protein.

Figure 4. Inverse relationships between the 24-h EE response to fasting and the average change in 24-h EE during overfeeding (A) and during low-protein overfeeding (B).

The 24-h EE response to fasting and to overfeeding is expressed as percent change compared to the 24-h EE measured during energy balance. The average change in 24-h EE during overfeeding was calculated as the mean value across the four overfeeding diets. The best-fit line is displayed in both panels. Vertical and horizontal lines indicate points with no change in 24-h EE compared to energy balance.

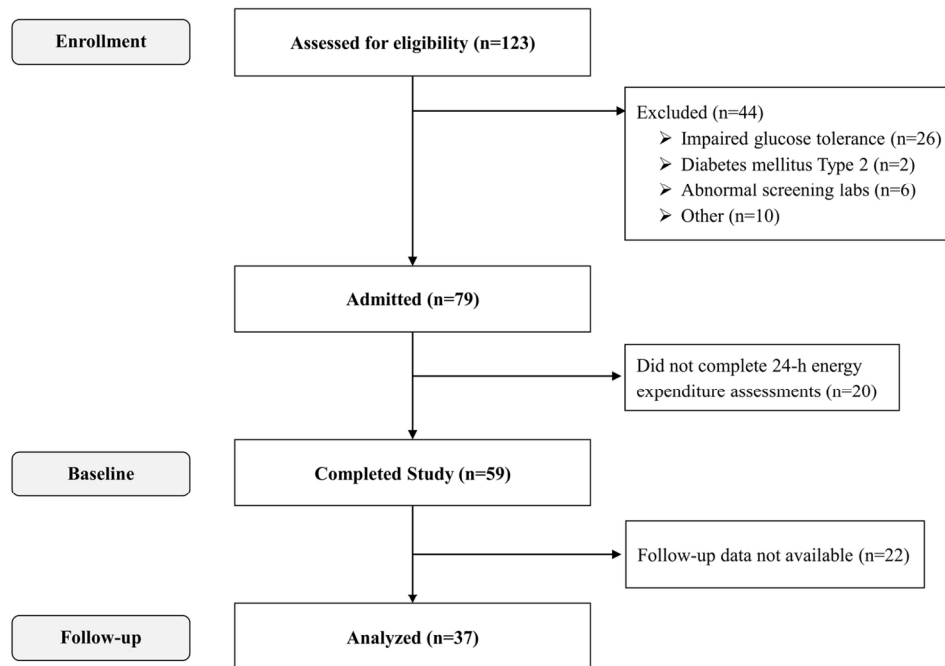
Figure 5. Associations between body weight change after 6 months and the 24-h EE responses to overfeeding and fasting.

Inverse associations between weight change after 6 months from the discharge and change in 24-h EE with fasting (Panel A) and during low protein overfeeding (Panel B). Positive relationship between the increase of 24-h EE with high carbohydrate overfeeding and weight change (Panel C, two high carbohydrate diets were excluded as less than 95% of food was consumed). Inverse relationship between RQ during 24-h of fasting and weight change (Panel D). The mean follow-up time was 6.5 ± 0.9 months with a weight change of 1.2 ± 4.2 kg (range: -6.1 to 11.2 kg). No point met the statistical criteria to be an outlier. All associations were still significant ($p < 0.05$) when excluding the subjects with the greatest weight change. Results for weight change expressed as a percent of baseline weight are the following: %EE response to fasting ($r = -0.36$, $p = 0.03$), low-protein overfeeding ($r = -0.51$, $p = 0.007$) and high-carbohydrate overfeeding ($r = 0.34$, $p = 0.05$); RQ during fasting ($r = -0.44$, $p = 0.006$).

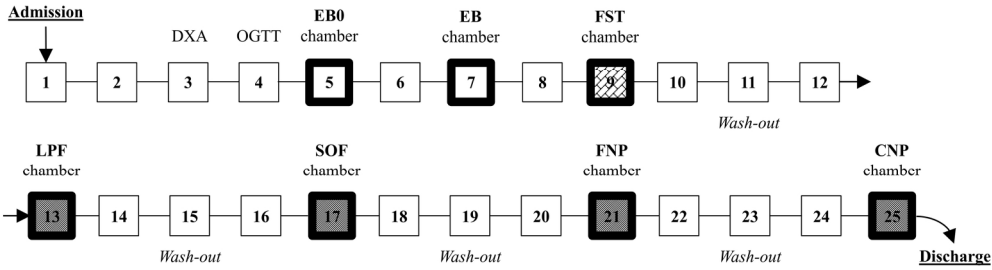
Figure 6. Phenotypes of 6-month weight change based on the 24-h EE responses to low-protein and high-carbohydrate overfeeding.

Subjects were categorized in four subgroups according to the median percent change in 24-h EE during low-protein and high-carbohydrate, normal-protein overfeeding (2 high carbohydrate diets were excluded as less than 95% of food was consumed). Subjects with a lower-than-median EE response during low-protein overfeeding and a higher-than-median response during high-carbohydrate, normal-protein overfeeding gained more weight as compared to those with the

opposing EE responses (mean difference= +7% of their baseline weight, $p=0.007$). The mean follow-up time was 6.5 ± 0.9 months with a weight change of 1.2 ± 4.2 kg (range: -6.1 to 11.2 kg).



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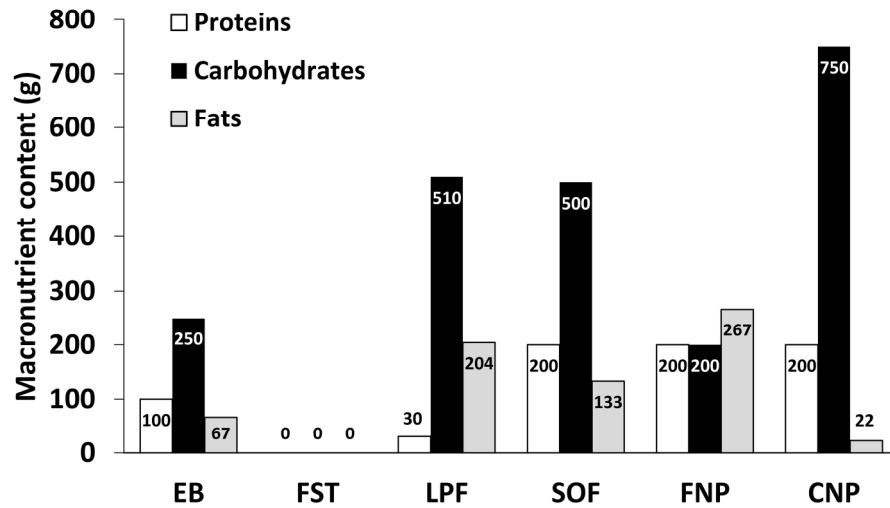


EB0 and EB: energy balance diet with 50% carbohydrate, 30% fat, 20% protein
FST: fasting
LPF: low-protein overfeeding with 51% carbohydrate, 46% fat, 3% protein
SOF: standard overfeeding with 50% carbohydrate, 30% fat and 20% protein
FNP: high-fat, normal-protein overfeeding with 20% carbohydrate, 60% fat and 20% protein
CNP: high-carbohydrate, normal-protein overfeeding with 75% carbohydrate, 5% fat and 20% protein

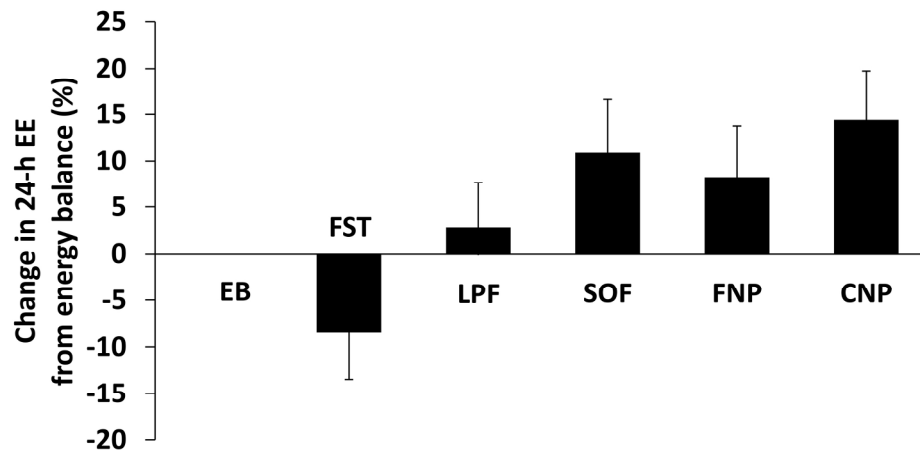
FST, LPF, SOF, FNP and CNP chambers were done in a random order.

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A



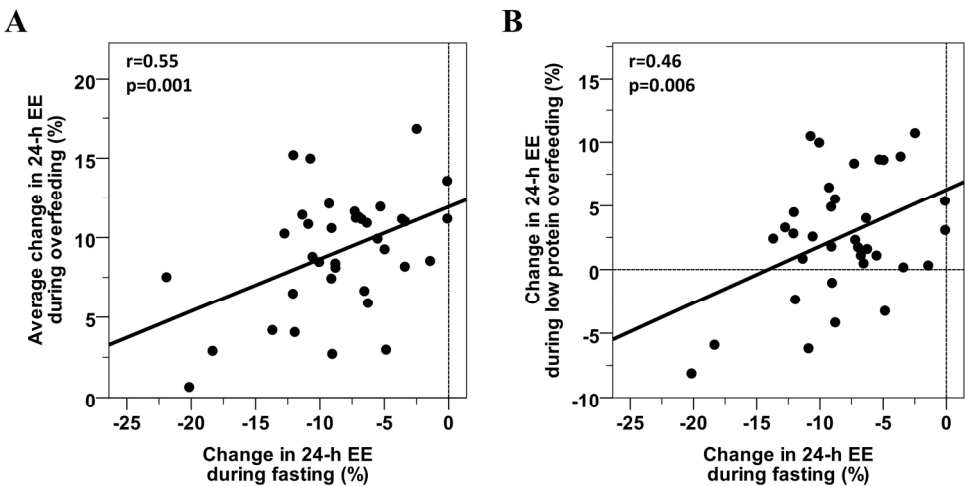
B



Protein, carbohydrate and fat content of the diets are expressed in grams based on a representative diet for an individual requiring 2000 kcal for energy balance and 4000 kcal for overfeeding (Panel A). The 24-h EE response to each dietary intervention is expressed as percent change compared to the 24-h EE measured during energy balance (Panel B). Error bars represent mean with SD.

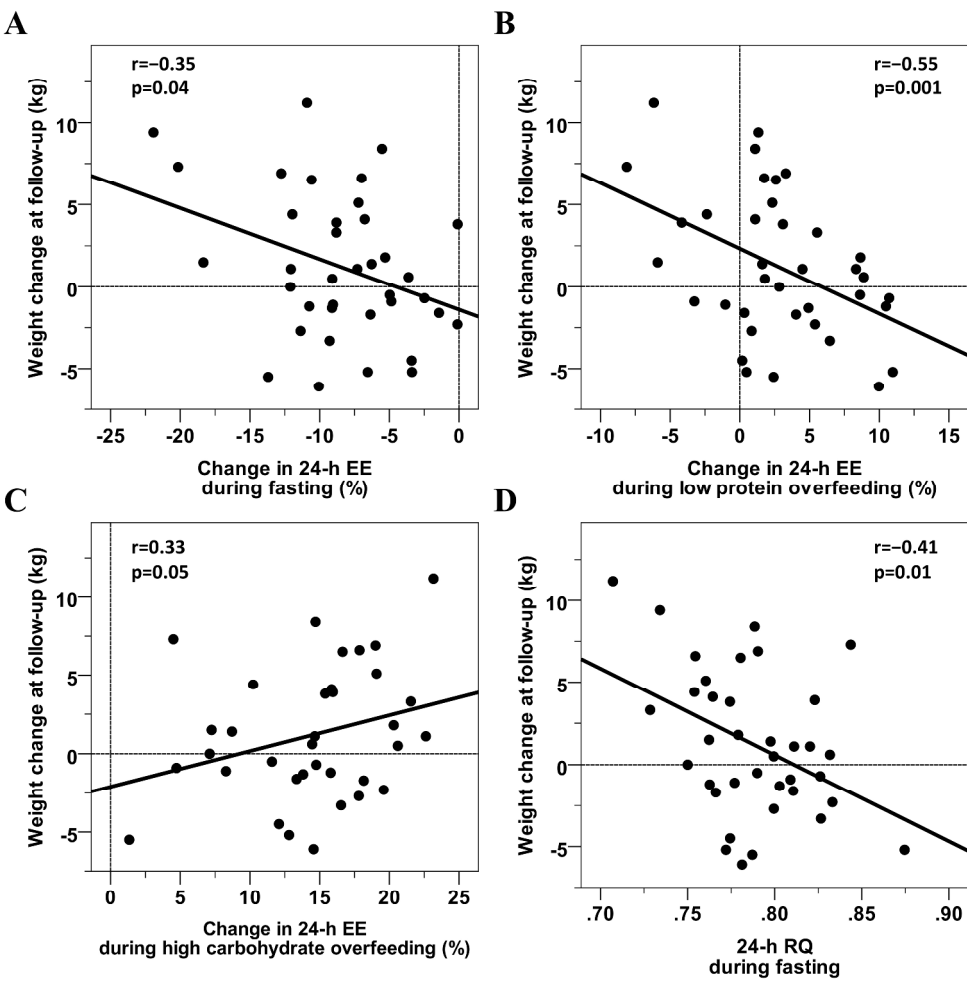
EB, energy balance; FST, fasting; LPF, low-protein overfeeding with 51% carbohydrate, 46% fat, 3% protein; SOF, standard overfeeding with 50% carbohydrate, 30% fat and 20% protein; FNP, high-fat, normal-protein overfeeding with 20% carbohydrate, 60% fat and 20% protein; CNP, high-carbohydrate, normal-protein overfeeding with 75% carbohydrate, 5% fat and 20% protein.

102x117mm (600 x 600 DPI)

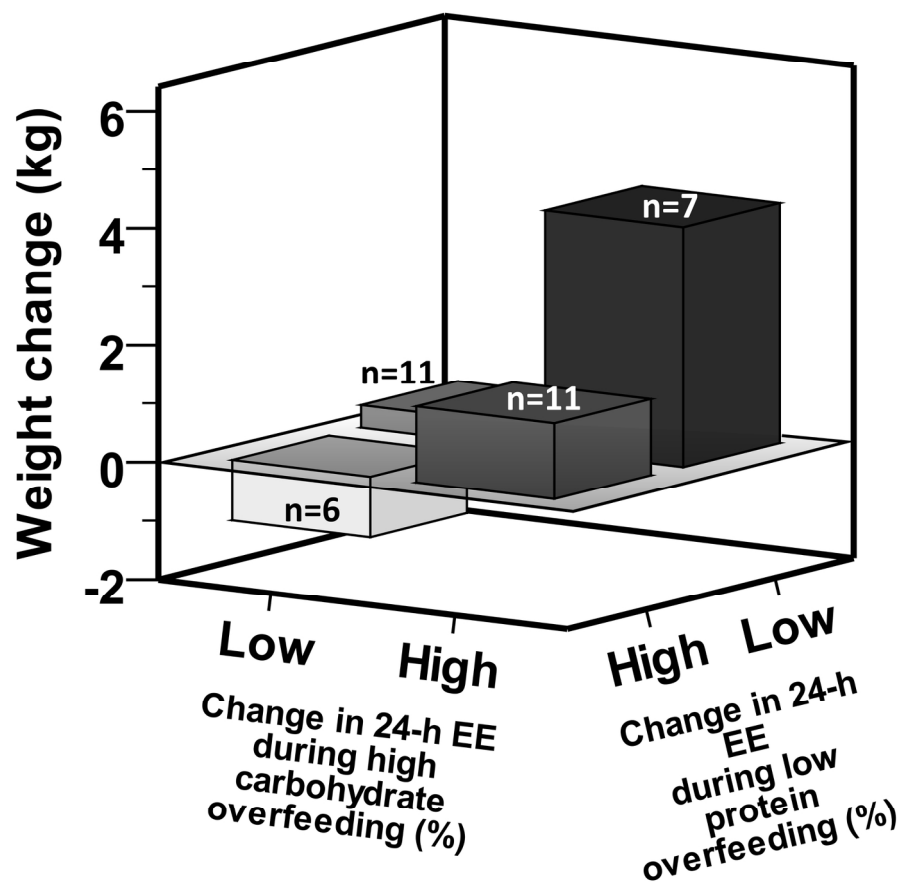


The 24-h EE response to fasting and to overfeeding is expressed as percent change compared to the 24-h EE measured during energy balance. The average change in 24-h EE during overfeeding was calculated as the mean value across the four overfeeding diets. The best-fit line is displayed in both panels. Vertical and horizontal lines indicate points with no change in 24-h EE compared to energy balance.

93x48mm (600 x 600 DPI)



182x185mm (600 x 600 DPI)



Subjects were categorized in four subgroups according to the median percent change in 24-h EE during low-protein and high-carbohydrate, normal-protein overfeeding (2 high carbohydrate diets were excluded as less than 95% of food was consumed). Subjects with a lower-than-median EE response during low-protein overfeeding and a higher-than-median response during high-carbohydrate, normal-protein overfeeding gained more weight as compared to those with the opposing EE responses (mean difference= +7% of their baseline weight, $p=0.007$). The mean follow-up time was 6.5 ± 0.9 months with a weight change of 1.2 ± 4.2 kg (range: -6.1 to 11.2 kg).
85x81mm (600 x 600 DPI)